NATIONAL URANIUM TAILINGS PROGRAM

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Canada

Investigation of Bacteria-Catalyzed Reactions Occurring in Uranium Mill Tailings At Low Oxygen Levels

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SUMMARY

The following report describes our approach to the investigation of bacteria-catalyzed reactions occurring in uranium mill tailings at low oxygen levels. Our objective was to characterize the bacteria found in anaerobic and near-anaerobic uranium mill tailings and determine what (if any) changes these bacteria caused to the nutrient and Radium-226 (Ra-226) and Lead-210 (Pb-210) concentrations in the eluted pore water.

The bacteria population in the uranium mill tailings was dominated by Gram negative rod-shaped bacteria. Although, there were several identifiable groups (using the Enterotube/Oxiferm tube system) the bacteria were very similar in their response to the tests in the Enterotube/Oxiferm tube identification system.

Changes in pH and the concentration of ferrous iron and phosphate demonstrated that the bacteria influenced the characteristics of the pore-water of the uranium mill tailings. However, over the duration of this experiment the chemical changes in the tailings was more significant and would have a greater impact on a model describing the interactions in the mill tailings.

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1.0 INTRODUCTION

This report was prepared by Dobrocky Seatech Ltd. and contains the experimental results and interpretations of the "Investigation of Bacteria-Catalyzed Reactions Occurring in Uranium Mill Tailings at Low Oxygen Levels". The project was completed by the scientists and staff at Dobrocky Seatech Ltd.'s Sidney, B. C. laboratories, with the help of subcontractual analysis for the Pb-210 and Ra-226 which were performed at the University of Waterloo under the direction of Dr. H. Sharma.

1.1 STATEMENT OF THE PROBLEM

The goal of the National Uranium Tailings Program (NUTP) is to create a predictive model describing the rate at which radioactive metal ions are lost from uranium mill tailings. Although considerable information exists about the activity of bacteria in aerobic environments, the effect of microorganisms on the mobility of metal ions in anaerobic or near-anaerobic environments is poorly understood.

Up to 10% of the Ra-226 present in uranium mill tailings can be leached from the mill tailings by water (Yagnik et al, 1981). The effect of microorganisms on the mobility of the Ra-226 will depend on the species present, their interaction and ultimately how they are able to change the mill tailings environment. Field observations indicate that under aerobic conditions, the bacteria increase the percentage of Ra-226 leached from the mill tailings (Butler, 1985). Therefore, the effect of microorganisms on the chemical environment will be an extremely important component of any models created to predict the rate radioactive metals (such as Ra-226) are lost from the tailings. In aerobic acidic environments, the problem is greatly simplified by the dominating influence of Thiobacillus ferrooxidans (Atlas and Bartha, 1981).

1.2 THE OBJECTIVE OF THE PROJECT

The objec ive in this project was to characterize the bacteria populating anaerobic and near-anaerobic uranium mill tailings and determine the effect(s) these bacteria had on the nutrient, Ra-226 and Pb-210 concentrations in eluted pore-water. Our working hypothesis was, the bacteria would decrease the rate at which Ra-226 and Pb 210 were leached from the tailings. To test this hypothesis the following tasks were performed;

- uranium mill tailings were collected from Rio Algom's Nordic Mine tailings site in Elliot Lake, Ontario;

- the permeability of the columns was determined;

- the tailings (collected in plastic core liners) were placed in the core elution apparatus designed by Dobrocky Seatech Ltd.;

- the tailings were then eluted with a chemically defined medium and the eluted pore-water was collected for nutrient, Ra-226 and Pb-210 analyses;

- for each treatment the population size of the bacteria was determined in a sample column;

- and the bacteria cultured from the columns were characterized using the Enterotube/Oxiferm rapid identification system.

Typically, T. ferrooxidans is associated with bioleaching and increased metal ion mobility (Atlas and Bartha, 1981). This bacterium is capable of oxidizing the metal-sulphides of the parent ore into metal-sulphates which are more easily extracted. T. ferrooxidans has specific growth requirements including temperature, pH, iron, nutrients and oxygen (Atlas and Bartha, 1981). If any of these requirements are not met the bacterium is unable to initiate growth.

The tailings heaps at Elliot Lake were capped with dolomite or vegetation, and the pH of the tailings pile range from 8 at the surface to 3 towards the middle. The pH at the interface between the aerobic and anaerobic zones in the tailings is 6 (Sharma, pers. comm.). Therefore, we would expect to find acidophilic or acidotrophic bacteria. The tailings typically have a low organic content (Kalin, 1983) which will tend to limit the presence of heterotrophic bacteria. Groudev et al. (1978) found thiobacilli, sulfur bacteria, iron bacteria (Gallionella and Leptrthrix), heterotrophs (Desulfovibrio, Bacillus and Pseudomonas) as well as moulds, yeast and algae in acidic mill tailings from a copper leaching operation. Two other species of microorganisms that may be present at a low partial pressure of oxygen in uranium mill tailings are Rhodotorula sp. (yeast) and T. acidophilus (Nekar et al., 1977). Both species are heterotrophic or facultatively heterotrophic bacteria and are able to tolerate a wide pH range (3.5 - 6); and low organic levels (Butler, 1985). Therefore, the evidence suggests that a large number of microorganisms may be found in the mill tailings and the microorganisms could have a significant impact on the chemistry of uranium mill tailings.

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2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL RATIONALE

Dobrocky Seatech Ltd., in cooperation with the University of Waterloo and Dr. H. Sharma, undertook a series of laboratory experiments designed to determine the effect of anaerobic and near-anaerobic conditions on bacterial-catalyzed reactions in uranium mill tailings.

We have designed the following experiments to determine the effect of microorganisms on the rate of radioactive metal efflux under anaerobic and near-anaerobic conditions. The data obtained from these experiments provide baseline information for the future development of models describing the rate of radioactive metal efflux from uranium mill tailings into the environment. The emphasis in these experiments was on the mobility of Ra-226. Analysis of lead-210 (Pb-210) was included in the experiments because it is known to be present, and its mobility in anaerobic tailing was not well understood.

The tailings material were obtained from Elliot Lake and transported to Dobrocky Seatech Ltd.'s laboratory in B.C. The mill tailings were collected in plastic coring-sleeves to minimize the impact of sampling and transportation on the microrganisms. Cores of the mill tailings were preferred over collecting the mill tailings and repacking the columns because the cores more closely mimic the tailings environment. Temperature, flow rate, nutrient content, pH and oxygen concentration of the water to be passed into the column was controlled. The pore water was eluted, collected and analyzed for nutrients and radioactive metal concentrations. Four columns mounted on the outside of the elution apparatus (Figure 2.1) were sampled for the number and species characteristic of the indigenous bacteria.

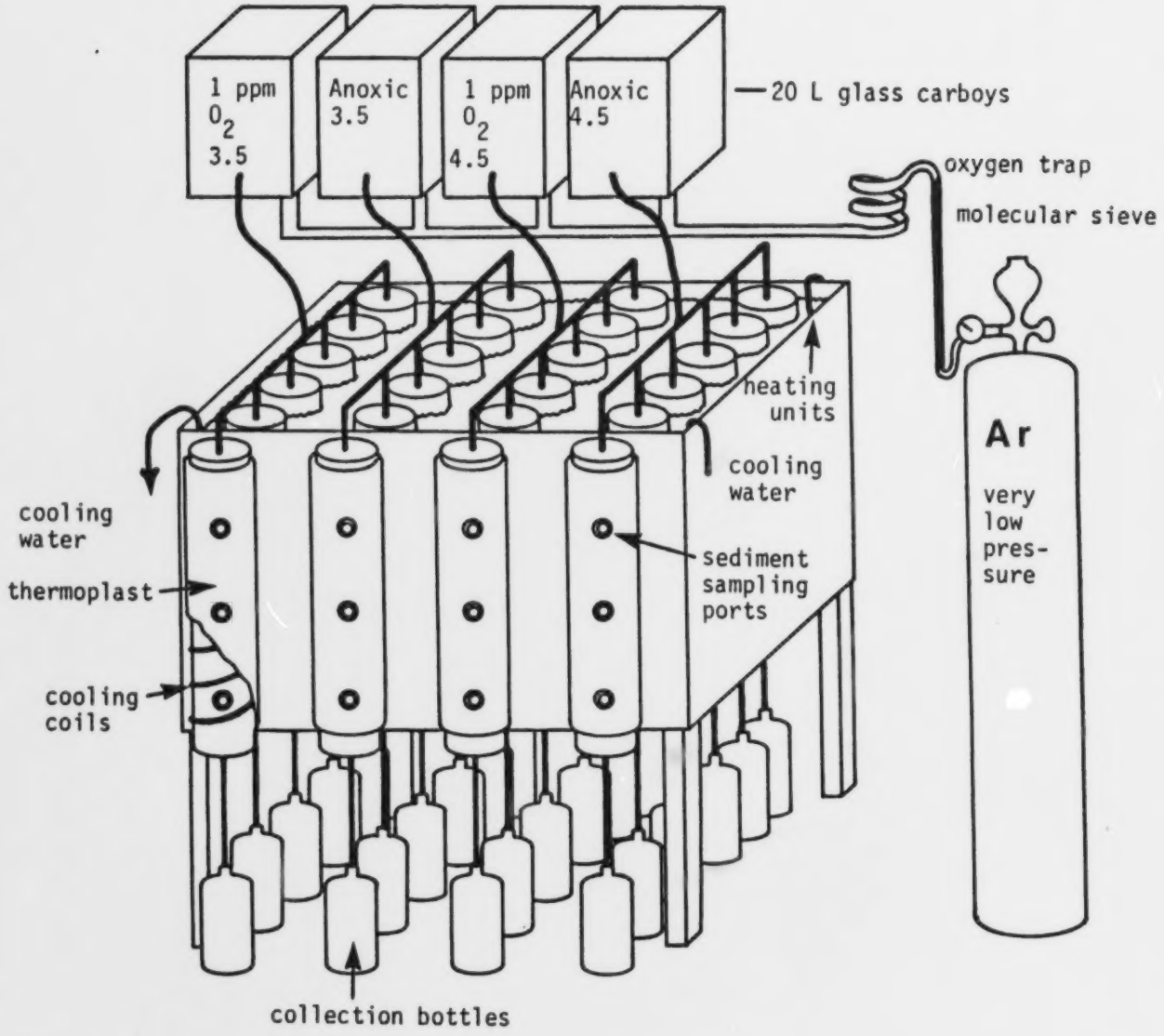

Figure 2.1 Experimental set-up.

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2.2 EXPERIMENTAL DESIGN

2.2.1 Collection of the Uranium Mill Tailings

The uranium mill tailings were collected from Rio Algom's Nordic Mine tailings site. Thirty cores were collected in 0.5 m plastic coring-sleeves, the tops of the cores were plugged with cotton fiber and sealed with plastic core caps and electrical tape. The cores were loaded into an aluminum box filled with packing material and air-freighted to Dobrocky Seatech Ltd.'s office in Sidney, B.C.

The uranium mill tailings were collected from approximately 1 m below the top of the tailings pile to a depth of 1.5 m.

2.2.2 Column Elution Apparatus

All the tubing and glassware to come into contact with the mill tailings was sterilized prior to being used in the experiment. All the glass and plasticware used for the nutrient analyses was acid washed prior to use in the nutrient analyses.

The uranium mill tailings were prepared for the elution apparatus (Figure 2.2) by replacing all the packing material from the top and bottom of the columns with glass wool and then sealing the bottoms and top of the columns with disinfected rubber stoppers. The stoppers were then taped to the column with duct tape. The mill tailings used in the sterile columns were removed from the columns, autoclaved for 30 min at 15 psi and then loaded into the clean, disinfected core-liners and sealed according to the procedure described above.

Sterile carboys (20 L) were mounted above the columns. The elution medium was allowed to flow from the carboys to the columns, using gravity feed. The flow rate of the columns was controlled from the output ports at the bottom of the columns. Argon gas was used to compensate for the changes in volume of the carboys over the duration of the experiment.

The constituents in the elution medium are described in Table 2.1 the arrangement of the individual columns is described in Table 2.2 and the length and the initial radioactivity of the columns are shown in Table 2.3.

The designations "Sterile", "Nonsterile" and "Bacterial" (Table 2.2) refer to the method used to prepare or sample the tailings. The "Sterile" tailings were removed from the plastic coreliners and sterilized in an autoclave (121°C at 15 psi) for 30 min. During this time the plastic liners were washed and disinfected with 10X strength Lysol disinfectant. The sterilized tailing were then transferred to the plactic coreliners and stoppered with sterile rubber stoppers. The "Nonsterile" tailings were not modified. The "Bacterial" columns were sampled for the number and identity of the bacteria in the uranium mill tailings. Holes were drilled 5 cm from the top, in the middle (25 cm from top) and 5 cm from bottom in each of the coreliner. The holes were plugged with sterile rubber stoppers.

2.2.3 Column Permeability Test

The permeability test was carried out using a falling-head permeameter (Figure 2.2). The test is conducted on the basis that water passing through the sample causes the water in the standpipe to drop from h_0 to h_1 in a measured period of time (t_1). Preparation for the test consisted of assembling the apparatus and pouring tap water into the standpipe until it permeated through the sample and overflowed the column. Once this was completed water in the standpipe was tapped to h_o, and as it fell, was measured (mm) every 0.5 H for 5 H. The permeability of the sample was calculated using three equations,

$$k \frac{h}{d} A = -a \frac{dh}{dt}$$

Then

$$k \frac{A}{d} \int_0^{t_1} dt = -a \int_{h_0}^{h_1} \frac{dh}{h}$$

from which

$$k = \frac{ad}{At_1} \log_e \frac{h_0}{h_1}$$

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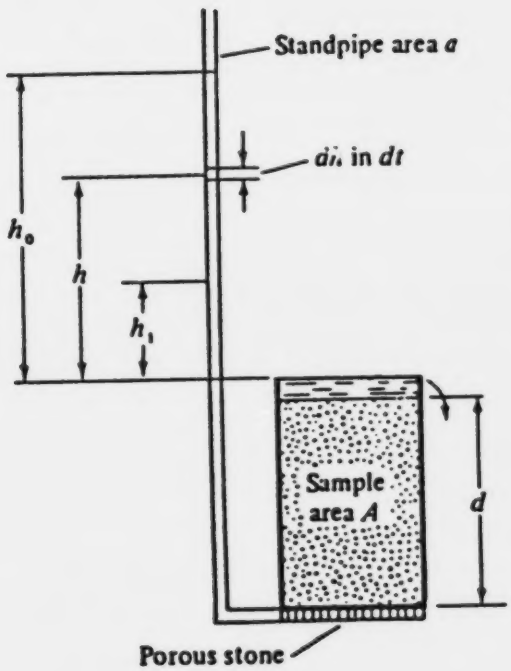

Figure 2.2 Falling-head permeameter.

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Table 2.1 Nutrient Contents of Elution Media.

pH[a]	(O_2) mg/L	(PO_4) mg/L	(NO_3) mg/L	(SO_4) mg/L	(Dextrose) mg/L
3.5	0[bc]	12.2	153.3	19.8	15.0
3.5	1	12.2	153.3	19.8	15.0
4.5	0	12.2	153.3	19.8	15.0
4.5	1	12.2	153.3	19.8	15.0

(a) ± 0.20 pH units.
(b) Winkler oxygen determination, detection limit of 0.05 ppm.
(c) ± 0.3 ppm is representative of the range used during the experiment.

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Table 2.2 Column Location and Identification

	Column Identification Numbers			
	pH = 3.5		pH = 4.5	
	1 ppm[a]	0 ppm	1 ppm	0 ppm
Sterile	2	25[b]	27	28
	29	1	14[b]	26[b]
Nonsterile	5	8	9	10
	11	15	16	17
	19	20	22	23
Bacterial	4	12	18	24

(a) Oxygen Concentrations in Elution Media.
(b) 1 Molar NaN_3 was used to ensure sterility of columns after the second samples were removed.

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Table 2.3 Column Bed Volume and Relative Measure of Radioactivity.

Column No.	Bed Volume (cm^3) x 10^3	Radiation (mRAD/hr)
1	1.9	0.2
25	1.6	0.15
4	1.7	0.1
5	1.7	0.2
8	1.7	0.2
9	1.8	0.2
10	1.9	0.25
11	1.8	0.2
12	1.9	0.2
14	1.6	0.15
15	1.8	0.15
16	1.7	0.2
17	1.8	0.1
18	1.9	0.2
19	1.7	0.2
20	1.7	0.2
22	2.1	0.15
23	2.1	0.1
24	1.7	0.02
25	1.9	0.015
26	1.9	ND[a]
27	1.5	0.01
28	2.0	0.02
29	1.7	0.02

(a) Radiation level approximately equal to background measurements.

2.2.4 Nutrient Analyses

Eluted material was collected over two week intervals into acid washed plastic bottles which had been filled with argon. The nutrient samples were then frozen until the analyses could be completed.

2.2.4.1 Orthophosphate

Orthophosphate was determined by a spectrophotometric method modified after McQuaker (1976). The procedure involves formation of heteropolymolybdophosphoric acid through reaction of the orthophosphate in the presence of ammonium molybdate under acidic conditions. The molybdophosphoric acid is reduced by ascorbic acid to form a 'molybdenum blue' complex which is measured colorimetrically at 885 nm. The presence of antimony increases the reaction rate and intensifies the blue colour.

The major interferences in the method are from high levels of arsenate and/or silicate. However, at the concentration of sulphuric acid used in the method, silicate interference is negligible.

Absorbance measurements were made using a Coleman 46 UV-VIS spectrophotometer. Calibration, using four standards and a blank, was carried out daily and sample values were calculated from the linear regression of these curves. All results were corrected for cell absorbance and instrument drift was minimized by using a blank reference cell containing Milli-Q deionized water. All measurements were made directly after zeroing the instrument with the reference cell.

The range of the method, using 2 cm path length cells was 0.002 - 0.4 mg/L phosphorus. Precision (as the relative coefficient of variation) at concentrations of 0.1 and 0.4 mg/L phosphorus was 0.78% and 0.27% respectively.

2.2.4.2 Iron (II)

Ferrous iron was determined spectrophotometrically (at 562 nm) by a modification of the ferrosine method introduced by Stookey (1970). Ferrosine (the disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine) reacts with divalent iron to form a water soluble magenta complex species which is stable in the range of pH 3-6.

Absorbance measurements were carried out using a Coleman 46 UV-VIS spectrophotometer. A calibration curve was prepared daily using the readings from four standards and a blank, and sample values were calculated from the regression lines produced from these curves.

The range of the method, using 2 cm path length cells was 0.017 to 1.0 mg/L Fe^{2+}.

Precision (as the relative coefficient of variation) at concentrations of 0.05 and 0.11 mg/L Fe^{2+} was 4.6% and 3.6% respectively.

2.2.4.3 Total Iron

The column eluate was analyzed for total iron by direct aspiration flame atomic absorption spectrophotometry (FAAS) using a Varian AA-1475 spectrophotometry and operating conditions outlined below.

Wavelength:	248.3 nm
Lamp current:	5.0 mA
Slit width	0.2 nm
Flame	C_2H_2/Air
Other:	Double beam mode with deuterium background correction

A working curve was established using a matrix blank and three calibration standards to cover the range of sample values encountered. The blank and standards were prepared using a 'media matrix' that had all the normal media constituents except iron added. The range of the method was 0.08 - 10 mg/L.

Precision (as the relative coefficient of variation) at concentrations of 0.5 and 5 mg/L Fe was 3.4% and 0.27% respectively.

2.2.4.4 Sulphate

Sulphates were determined by a modified turbidimetric method described by McQuaker (1976). The sulphate present in the samples is precipitated as barium sulphate under acidic conditions. The turbidity is then measured using a Coleman UV-VIS spectrophotometer at 420 nm.

A working curve was established daily using a matrix blank and four calibration standards over a range of 2-30 mg/L. The detection limit of the method was 0.7 mg/L SO_4. All samples had to be diluted 50-fold in order to fall within the working curve of the method. The calibration curve was entered into a linear regression program from which sample values were calculated.

Precision (as the relative coefficient of variation) at concentrations of 2 and 30 mg/L SO_4 was 13% and 2.4% respectively.

2.2.4.5 Nitrate

The method used to determine nitrate was based on the method described by Hach Company, 1984. The method is a modification of the cadmium reduction method in which gentisic acid replaces 1-napthylamine. All reagents are combined in a single stable powder (Nitra Ver 5 nitrate reagent) which can be directly added to samples that have been pre-treated with hydroxylamine hydrochloride to remove any interferring iron III present. The resulting amber-coloured solution is measured colorimetrically at 500 nm using a Coleman 46 UV-VIS spectrophotometer.

Four calibration standards (and a blank) were prepared daily from stock nitrate solution. A 'media matrix' containing all normal media constituents, except nitrate, was used for dilution. A linear regression curve was used to calculate sample concentrations and all results were corrected for cell absorbance and instrument drift using a blank reference cell containing Milli-Q deionized water.

The range of the method was 0.4-40 mg/L using a 1 cm path length cell. Precision (as the relative coefficient of variation) at concentrations of 10 and 30 mg/L was 1.9% and 3.5% respectively.

2.2.4.6 pH

The pH of the eluate from each column of tailings was determined with a Fisher Accumet pH meter (Model 291). The pH of samples of the tailings in the bacterial columns was also determined. The tailing were suspended in distilled water and the pH measured with the pH meter.

2.2.5 Methods Used for Bacterial Analyses

Four columns (one for each treatment) had holes drilled in the top, middle and bottom of the columns. These holes (ports) provided an entry point to sample the bacterial population size and characteristics during the experiment. The bacteria were sampled prior to beginning the experiment and then every second week for the following eight weeks.

Approximately 1 g of sediment was removed with a sterile glass coring device. The coring device and glass rod were placed in a dilution bottle containing 99 mL of sterile distilled water. The bottle was then vigorously shaken and a 1 mL sample was transferred to another bottle containing 99 mL of sterile distilled water. A dilution series was then similarly prepared over the range 10^{-2} to 10^{-5} dilutions. The bacteria were initially grown on nutrient agar plates under an Argon environment. The colonies were allowed to grow over 48 hr at which time

the number of colonies were determined. The colonies on the plates were then examined for the variety of coloration. The colonies' Gram reaction, cell shape and oxidase reaction (Oxidase reagent, Hoffman-La Roche) were determined. All the colonies were subjected to a series of biochemical tests by inoculating Enterotube II (Hoffman-La Roche) with samples of each colony. All oxidase positive colonies were also used to inoculate Oxi-Ferm tubes (Hoffman-La Roche). The tubes were examined after 24 and 48 hr at which times the reactions were recorded and the indol and Voges-Proskauer test were made, also using chemicals provided by Hoffman-La Roche. Tentitive identification of the bacteria was made whenever possible.

3.0 RESULTS AND DISCUSSION

3.1 EXPERIMENTAL DESIGN

The design of the experiment and apparatus used for the study of the effects of bacteria in uranium mill tailings was novel and warrants a brief discussion describing some of the successes and difficulties experienced during the experiment.

The experimental design required individual cores to be taken from approximately 1 m below the surface of an older (10-15 yr) pile of uranium mill tailings. It was hoped that this would provide near-anaerobic or anaerobic tailings. The amount of ferrous iron measured in the eluted pore water of the columns treated with 0 ppm elution media suggest that the mill tailings were approaching anoxia but were probably not anaerobic.

The elution medium was introduced to the top of each column and allowed to free flow through the column. The flow rate of the elution medium to and from each column was controlled. During the experiment the flow rate through some of the columns approached zero, due to compaction of the tailings in the columns by the input media and the small particle size of the uranium mill tailings. Some of the columns were reactivated by pumping the pore water from the bottom of the columns. As well, the flow rate through the columns was variable, as demonstrated by the range of total elution volumes collected during the experiment (Table 3.1). In future experiments, both these problems could be reduced by using a pump to draw the elution medium through the column at a predetermined flow rate.

Placing the columns in a water tank provided a good temperature control (9.3 ± 1.2°C) of the uranium mill tailings during the experiment.

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The method used to input the elution medium into each column proved to be adequate. However, it could be improved by the use of separate feedlines directly from the reservoir and the addition of a thistle tube on each tube to prevent cross contamination.

Jacketed columns located outside the cooling tanks were used in an experiment designed to sample the uranium mill tailings for indigenous bacteria (top, middle and bottom). The design of the apparatus was excellent for this task. However, there was some concern that oxygen entered the mill tailings through the sampling-ports during the sampling procedure. This may have affected the results as the microenvironment of the area around the sampling ports were more aerobic and uncharacteristic of the unsampled columns.

There was some concern that the sterile columns had become contaminated during the experiment. Therefore, some of the sterile columns were treated with 1 M NaN_3 (a respiratory inhibitor) to inhibit the activity of any bacteria that may have entered the column. However, the addition had no observable effect on the nutrient concentrations in the water eluted from the uranium mill tailings. The inability to discern differences between the azide treated and untreated mill tailings suggest that our fears may have been unfounded.

3.2 PERMEABILITY TEST

The falling head permeability of a column filled with uranium mill tailings was determined to be 0.94 ± 0.073 cm/hr. Although this was a good estimate of the initial permeability of the uranium mill tailings, the permeability changed over time. This problem could be eliminated with the use of a pump to draw the pore-water from the tailings at a known flow rate. However, the decreasing flow rate may be representative of the real environmental situation and will have to be taken into consideration when modelling the rate ground and rain water pass through the uranium mill tailings.

Table 3.1 Total Volume Eluted from the Uranium Mill Tailings

Treatment	Volume of Samples (mL) pH = 3.5 0 ppm[a]	pH = 3.5 1 ppm[a]	pH = 4.5 0 ppm[a]	pH = 4.5 1 ppm[a]
Sterile	1949.00	1159.00	3981.00	3585.00
	3685.00	2731.00	1558.00	3596.00
Nonsterile	2912.00	2303.00	4387.00	1701.00
	3461.00	3771.00	2919.00	3150.00
	3580.00	3471.00	1981.00	2318.00
Bacterial	1928.00	1042.00	1419.00	1884.00

(a) = Oxygen Concentration of Elution Medium

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3.3. NUTRIENT ANALYSES

3.3.1 pH of Eluted Pore-Water

The pH of the eluted pore-water was determined for each of the columns containing uranium mill tailings, pH and averaged values are tabulated in Table 3.2. There was no discernible difference between the nonsterile and bacterial columns. Therefore, the reported pH of the eluted pore-water represent the combined average of nonsterile and bacterial tailings.

The pH of the eluate collected from the sterile columns exposed to 1.0 ppm oxygen at pH 3.5 was similar to the tailings exposed to 1 ppm oxygen at pH 4.5. The pH of the eluate increased from approximately 4.5 to 6.5 during the experiment. The pH of the eluate collected from the sterile columns exposed to an elution medium containing no oxygen at a pH of 3.5 was between 3.0 and 3.5. The sterile tailings exposed to an elution medium containing no oxygen at pH 4.5 had an initial pH of 3.6 which increased to 4.4 over the duration of the experiment. The pH of the water eluted from the nonsterile and bacterial columns exposed to media containing 1 ppm of oxygen had an initial pH of approximately 3.3 which increased to approximately 4.5 over the duration of the experiment. This was a smaller increase in pH than observed in the eluate collected from the sterile tailing exposed to 1.0 ppm oxygen. The eluate collected from the nonsterile and bacterial tailings exposed to media containing no oxygen had an initial pH of approximately 3.7 which tended to increase towards the sixth week of the experiment and then decreased in the samples collected on the eighth week of the experiment.

These data indicate that the oxygen content of the solution passing through the tailings influenced the pH of the eluate (i.e., the observed difference in pH between the sterile tailings exposed to the media at 1 and 0 ppm oxygen, respectively). The bacteria present in the non-sterile and bacterial columns tended to cause a reduction in the pH of the columns exposed to oxygen, as compared to the sterile tailings treated in a similar manner.

Table 3.2 pH of Column Eluates

Treatment	Week Number	pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
		Average pH			
Sterile	2	4.97	3.06	4.37	3.61
	4	4.52	3.44	4.59	NC
	6	5.72	3.64	5.85	3.51
	8	6.25	3.52	6.44	4.42
Non-sterile	2	3.26	3.77	3.30	3.62
	4	3.68	3.85	3.91	3.92
	6	3.93	4.23	6.97	4.57
	8	4.59	3.66	4.80	3.64

(a) = Oxygen Concentration of Elution Medium
NC = No sample Collected

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Tailings sediment collected from each of the bacterial columns was suspended in a solution of distilled water. The pH of this suspension (uranium mill tailings slurry) was then determined and used to estimate the pH of the mill tailings (Table 3.3). The initial pH of the slurries ranged from 6.1 to 6.5. The pH of the tailings declined throughout the duration of the experiment to a pH of between 5.18 to 5.88. These data suggest that the solution used to elute the pore-water in the column was gradually stripping the tailings sediment of its buffering capacity. This may explain the observed increase in the pH of the eluted pore-water from the sterile tailings exposed to 1 ppm oxygen. However, a similar decrease in the pH of sterile tailings' slurries exposed to media containing no oxygen, resulted in only small increases in the pH of the corresponding eluted pore-water. Therefore, although the tailings sediment may have buffering capacity and contribute to the pH of the eluate, the pH will also depend on oxygen content and the activity of the bacteria.

From the above results and discussion, it appears that the bacteria indigenous to the aerobic mill tailings caused a reduction in the pH of the mill tailings, typical of the effects that might be caused by the respiratory activity of bacteria. However, the buffering capacity of the tailings sediment and the presence or absence of oxygen in the pore-water of the tailings also had a significant effect on the pH of the eluate.

3.3.2 Analysis for Ferrous Iron in the Pore-Water Samples

Prior to discussing the data a short description of the methods used to present each of the following nutrients is given in order to assist the reader.

The concentration of each nutrient and metal on the eluates was located in the table according to the columns location as specified in Table 2.2. Therefore, the ferrous concentration recorded in the first position of Table 3.4 (0.95 mg) is directly comparable to the ferrous concentration in the first position of Table 3.5 (0.01 mg), both measurements representing the ferrous iron concentration in column #2 after the first and second sampling, respectively. This method of presenting the data was adopted to help the reader examine the trends associated with treatment and those associated with individual columns.

Table 3.3 pH of Uranium Mill-Tailing Slurries From Bacteriological Columns

Position in Tailings Column	Week Sampled	pH = 3.5		pH = 4.5	
		1 ppm[a]	0 ppm[a]	1 ppm[a]	0 ppm[a]
Top	0	6.40	6.42	6.58	6.32
	2	5.76	5.58	5.63	5.52
	4	5.78	5.93	5.75	5.46
	6	6.00	6.00	6.52	6.01
	8	5.88	5.27	5.35	5.76
Middle	0	6.20	5.91	6.36	6.09
	2	5.54	5.67	5.68	5.52
	4	5.88	5.76	5.86	5.72
	6	5.85	5.84	6.01	5.84
	8	5.35	5.31	5.36	5.78
Bottom	0	6.25	6.38	6.13	6.14
	2	5.44	4.49	5.60	5.62
	4	5.94	5.89	5.65	5.39
	6	5.95	6.10	5.84	5.68
	8	5.18	5.28	5.58	5.45

(a) = Oxygen Concentration of Elution Medium

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In analyzing the data, it was observed that the nutrient values determined in the nonsterile eluates were dissimilar from those observed in the bacterial eluates of the same treatments. This was unexpected because other than the sampling ports added to the bacterial column the tailings were treated identically. At this time, the reason for these differences are unknown. However, several explanations have been postulated. The differences may have arisen due to the sampling variation between the tailings. As already noted, the variation between samples of tailings was significant, and the reason little conclusive evidence of the effect of bacteria on the mill tailings under anaerobic conditions could be obtained. In addition, the periodic sampling of the sediments in the bacterial columns may have increased the oxygen concentrations in the uranium mill tailings in isolated zones around the openings. The problem of air input into the uranium mill tailings could be eliminated or at least reduced by incorporating a self-sealing sampling port instead of the rubber stoppers.

More ferrous iron was eluted from the anaerobic mill tailings than from the aerobic mill tailings (Tables 3.4, 3.5, 3.6, 3.7). By the third sample (6 weeks into the experiment) the amount of ferrous iron eluted from the mill tailings was approaching zero (Table 3.6). There tended to be more iron eluted from the sterile anaerobic mill tailings than those containing bacteria (Table 3.4), which was indicative of bacterial activity acting on the ferrous iron. However, these differences were not observed after the second sampling (4th week of the experiment). Summing the measured ferrous iron concentration over the duration of the experiment (Table 3.8) demonstrates the above observations more dramatically.

Ferrous hydroxides are able to scavenge some (e.g. uranium) radiocations from the mill tailings. Therefore, a reduction in the concentration of available ferrous iron, caused by the bacteria in the anaerobic mill tailings, may have a significant impact on the rate radiocations are leached from the mill tailings.

Table 3.4 Ferrous Iron Analyses from First Sample

Treatment	Total Fe^{2+} Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.95	2.26	0.89	9.78
	0.02	4.57	1.62	2.82
Nonsterile	0.14	0.02	0.49	0.25
	0.31	1.01	0.16	0.08
	0.36	1.81	1.87	1.44
Bacterial	0.15	0.29	0.02	3.60

Table 3.5 Ferrous Iron Analyses from Second Sample

Treatment	Total Fe^{2+} Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.010	3.300	0.050	NC
	NC	0.068	0.145	NC
Nonsterile	NC	0.206	0.017	0.068
	0.138	0.353	0.103	0.053
	NC	0.392	1.746	NC
Bacterial	0.084	0.017	0.162	0.015

NC = No Sample Collected

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Table 3.6 Ferrous Iron Analyses from Third Sample

	Total Fe^{2+} Eluted (mg)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	0.050	ND	0.027
	ND	0.082	NC	ND
Nonsterile	0.092	0.005	ND	0.012
	ND	ND	0.004	0.003
	0.051	0.071	NC	0.088
Bacterial	NC	NC	NC	0.009

NC = No Sample Collected
ND = Below Detection of Method

Table 3.7 Ferrous Iron Analyses from Fourth Sample

	Total Fe^{2+} Eluted (mg)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	NC	ND	0.193
	ND	ND	NC	0.115
Nonsterile	0.040	0.001	ND	NC
	0.058	ND	ND	0.037
	ND	0.022	0.083	0.017
Bacterial	NC	NC	0.192	NC

NC = No Sample Collected

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Table 3.8 Total Ferrous Iron Leached from the Uranium Mill Tailings

Treatment	Ferrous Iron Concentration (mg) pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
Sterile	0.96	5.61	0.94	10.00
	0.02	4.72	1.64	3.27
Nonsterile	0.27	0.23	2.14	0.33
	0.51	1.36	0.27	0.17
	0.41	2.30	3.63	1.55
Bacterial	0.23	0.31	0.18	3.62

(a) = Oxygen Concentration of Elution Medium

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3.3.3 Concentration of Iron in the Pore-Water

The total iron concentration determined by AA spectrophotometry in the eluted pore-water was extremely variable both within and between treatments in the first two sets of samples (2nd and 4th week of the experiment; Tables 3.9 and 3.10). However, there was a marked decrease in the total iron after the second set of samples (Tables 3.11 and 3.12). The variability between columns of the same treatment prevent any meaningful comparisons between treatments. Summing the concentrations determined for each column of mill tailings over the duration of the experiment did not decrease the sample variation (Table 3.13).

3.3.4 Nitrate Analyses of the Pore-Water

The analysis of the nitrate concentrations in the eluate showed no obvious trends between treatments (Tables 3.14, 3.15, 3.16 and 3.17). This may have been due to the amount of variability between the columns of mill tailings. No trends were observed by summing the nitrate concentration of the eluted pore-water over the duration of the experiment (Table 3.18).

3.3.5 Sulphate Analyses of the Pore-Water

The sulphate concentration observed in the eluted pore-water were high throughout the duration of the experiment (Tables 3.19, 3.20, 3.21, 3.22). In general, more sulphate was found in the pore-water of the mill tailings treated with an elution media of pH 3.5 (Table 3.20). However, this trend was not supported by the summing of the sulphate eluted from the columns over the duration of the experiment (Table 3.23). This was probably due to the extreme concentrations of sulphate added to the pore-water from the mill tailings.

3.3.6 Analysis of Phosphate Concentrations in the Pore-Water

The concentration of phosphate observed in the pore-water varied dramatically (Tables 3.24, 3.25, 3.26, 3.27). However, summing the phosphorus eluted from each column of mill tailings over the duration of

Table 3.9 Total Iron - Analyses from First Sample

Treatment	Total Iron Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	2.07	52.09	1.44	52.27
	0.11	3.86	1.41	3.56
Nonsterile	0.90	131.33	7.11	0.32
	1.66	117.22	2.64	0.66
	1.43	5.57	271.95	251.08
Bacterial	2.28	0.07	12.24	158.70

Table 3.10 Total Iron - Analyses from Second Sample

Treatment	Total Iron Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.03	29.04	ND	NC
	NC	0.74	0.27	NC
Nonsterile	NC	3.58	ND	0.23
	1.71	4.12	0.17	0.35
	NC	4.78	12.74	NC
Bacterial	0.88	0.60	5.52	0.09

NC = No Sample Collected
ND = No Iron Detected

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Table 3.11 Total Iron - Analyses from Third Sample

Treatment	Total Iron Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	1.87	ND	1.14
	ND	0.11	NC	0.04
Nonsterile	3.16	0.36	0.49	0.33
	0.09	0.11	ND	0.54
	0.04	0.40	3.41	3.06
Bacterial	NC	NC	NC	0.13

NC = No Sample Collected
ND = No Iron Detected

Table 3.12 Total Iron - Analyses from Fourth Sample

Treatment	Total Iron Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	1.98
	ND	ND	NC	0.17
Nonsterile	3.85	0.47	ND	NC
	1.65	ND	ND	0.87
	0.51	0.36	3.78	1.31
Bacterial	NC	NC	7.67	NC

NC = No Sample Collected
ND = No Iron Detected

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Table 3.13 Total Iron Leached from the Uranium Mill Tailings

Treatment	Iron Concentration (mg) pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
Sterile	2.10	87.71	1.44	55.39
	0.11	4.71	3.12	4.65
Nonsterile	7.91	257.19	7.11	0.88
	5.11	121.45	2.81	2.42
	1.98	11.11	294.69	255.45
Bacterial	3.16	0.67	25.43	158.92

(a) = Oxygen Concentration of Elution Medium

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Table 3.14 Nitrate Analyses from First Sample

Treatment	Total Nitrate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	46.11	ND	35.55	37.09
	36.84	17.57	5.21	40.01
Nonsterile	17.09	1.90	31.60	11.00
	15.87	16.55	16.88	7.78
	34.32	27.22	0.60	9.79
Bacterial	20.19	11.79	ND	12.70

Table 3.15 Nitrate Analyses from Second Sample

Treatment	Total Nitrate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	23.95	ND	54.24	NC
	NC	20.43	53.30	NC
Nonsterile	NC	50.10	36.40	16.38
	69.77	54.24	29.31	57.55
	NC	NR	13.45	NC
Bacterial	70.56	11.92	2.17	13.87

ND = Below Detection of Method
NC = No Sample Collected
NR = No Value Reported

Table 3.16 Nitrate Analyses from Third Sample

	Total Nitrate Eluted (mg)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	ND	ND	20.61
	13.13	17.21	NC	16.70
Nonsterile	17.55	7.34	NC	18.45
	15.23	12.21	0.25	21.58
	20.37	10.59	NC	12.13
Bacterial	NC	NC	NC	15.17

ND = Below Detection of Method
NC = No Sample Collected

Table 3.17 Nitrate Analyses from Fourth Sample

	Total Nitrate Eluted (mg)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	NC	ND	12.28
	33.25	11.70	NC	NC
Nonsterile	31.16	5.40	3.25	NC5
	28.96	4.28	27.06	34.59
	25.07	6.90	20.01	6.03
Bacterial	NC	NC	16.01	NC

ND = Below Detection of Method
NC = No Sample Collected

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Table 3.18 Total Nitrate Leached from the Uranium Mill Tailings

Treatment	Nitrate Concentration (mg) pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
Sterile	70.06	0.00	89.79	69.98
	83.22	66.91	58.51	56.71
Nonsterile	65.80	64.74	71.52	45.83
	129.83	87.28	73.50	121.50
	79.76	44.71	34.06	27.95
Bacterial	90.75	23.71	18.18	41.70

(a) = Oxygen Concentration of Elution Medium

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Table 3.19 Sulphate Analyses from First Sample

Treatment	Total Sulphate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	683.70	727.70	904.96	1466.82
	1028.45	1018.77	453.27	1067.04
Nonsterile	990.93	677.32	884.80	566.82
	669.30	1337.63	759.60	217.84
	1144.00	725.76	913.10	1131.90
Bacterial	719.96	302.17	214.20	1481.20

Table 3.20 Sulphate Analyses from Second Sample

Treatment	Total Sulphate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	312.33	297.00	808.86	NC
	NC	344.64	435.54	NC
Nonsterile	NC	716.88	674.05	436.80
	4035.60	1140.70	399.04	829.60
	NC	845.04	410.64	NC
Bacterial	1575.00	198.72	114.26	154.44

NC = No Sample Collected

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Table 3.21 Sulphate Analyses from Third Sample

Treatment	Total Sulphate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	316.92	886.89
	542.85	751.23	NC	416.85
Nonsterile	393.71	257.25	NC	538.35
	453.71	187.28	88.92	434.23
	542.85	516.90	NC	631.53
Bacterial	NC	NC	NC	691.20

NC = No Sample Collected

Table 3.22 Sulphate Analyses from Fourth Sample

Treatment	Total Sulphate Eluted (mg) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	1221.00	1991.00
	661.10	263.14	NC	274.51
Nonsterile	1015.30	347.20	450.45	NC
	539.00	577.70	485.10	1132.06
	1199.00	467.16	1071.00	212.36
Bacterial	NC	NC	1837.50	NC

NC = No Sample Collected

Table 3.23 Total Sulphate Leached from the Uranium Mill Tailings

Treatment	Sulphate Concentration (mg) pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
Sterile	1539	1024	3753	4345
	2231	2378	889	1759
Nonsterile	2398	1998	2009	1542
	5696	3244	1733	2614
	1855	2555	2394	1976
Bacterial	2294	501	2166	2326

(a) = Oxygen Concentration of Elution Medium

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Table 3.24 Phosphate Analyses from First Sample

Treatment	Total Phosphate Eluted (ug) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	286.20	11.49	51.71	32.03
	ND	36.30	4.90	ND
Nonsterile	7.86	2.07	ND	5.25
	ND	12.00	6.54	ND
	8.87	3.24	0.25	ND
Bacterial	ND	4.57	3.21	50.78

Table 3.25 Phosphate Analyses from Second Sample

Treatment	Total Phosphate Eluted (ug) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	ND	4.851	189.564	NC
	NC	1.292	1.970	NC
Nonsterile	NC	5.109	1.970	1.229
	4.779	1.970	1.926	2.904
	NC	ND	107.616	NC
Bacterial	1.995	1.339	1.064	ND

NC = No Sample Collected
ND = Below Detection of Method

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Table 3.26 Phosphate Analyses from Third Sample

Treatment	Total Phosphate Eluted (ug) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	ND
	376.215	ND	NC	143.955
Nonsterile	18.844	ND	NC	ND
	ND	ND	0.456	ND
	ND	ND	NC	ND
Bacterial	NC	NC	NC	5.940

NC = No Sample Collected
ND = Below Detection of Method

Table 3.27 Phosphate Analyses from Fourth Sample

Treatment	Total Phosphate Eluted (ug) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	379.390	ND
	464.090	2.631	NC	ND
Nonsterile	ND	ND	8.715	NC
	172.920	4.081	502.810	ND
	147.950	ND	40.635	4.356
Bacterial	NC	NC	1.155	NC

NC = No Sample Collected
ND = Below Detection of Method

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the experiment did suggest some trends (Table 3.28). More phosphate tended to be leached from the aerobic mill tailings. There also appeared to be less phosphate in the pore-water of non-sterile mill tailings' columns (Table 3.28). However, the observation that less than 2% of the phosphate input at the top of the column was collected in the eluted pore-water was more dramatic. Therefore, even though an effect on the amount of phosphate leached from the mill tailings can be ascribed to the bacteria, the effect of the bacteria was small when compared to the chemical interactions that took place to remove the phosphate from the uranium mill tailings. This was possibly due to the precipitation of the phosphate with free metal ions. In addition, the solubility of phosphate complexed to some radiocations is dependent on the oxidation state of the metal, which would also effect the precipitation of the phosphate.

3.4 BACTERIA PRESENT IN THE URANIUM MILL TAILINGS

3.4.1 Characterization of the Bacteria

The Enterotube II and Oxi/ferm rapid identification systems were designed primarily for medical purposes. However, they have been successfully used to identify the bacteria from some environmental samples. The series of biochemical tests used by the Enterotube/Oxiferm rapid identification system was useful in determining (distinguishing) a series of recognizable groups of bacteria in the uranium mill tailings. The most striking observation made during the experiment was the similarity between all the bacteria. In general, the bacteria were Gram negative, rods (1.5-2.5 um long and 0.5 to 0.75 um wide), ca. 75% were able to metabolize citrate, ca. 45% able to metabolize urea. Over the duration of the experiment the number of isolates able to metabolize arabinose and the number of oxidase positive bacteria increased. The number of recognizable units of bacteria varied from 6 in week four to 13 in week six (Figure 3.1). The bacteria collected from the tailings showed no evidence of being able to produce H_2S, although some of the isolates were able to ferment glucose.

According to identification based on the Enterotube II and Oxi/ferm identification systems the tailings were initially dominated by Acinetobacter sp. and in later samples by Pseudomonas sp. Both of these

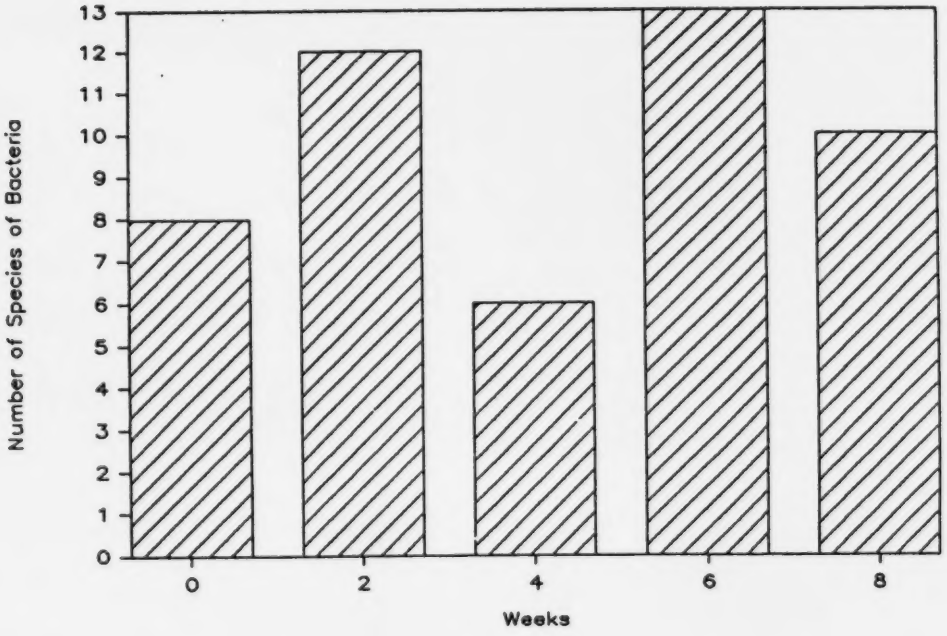

Figure 3.1 The number of different bacteria collected from the uranium mill tailings.

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Table 3.28 Total Phosphate Leached from the Uranium Mill Tailings

Treatment	Phosphate Concentration (ug) pH = 3.5 1 ppm[a]	pH = 3.5 0 ppm[a]	pH = 4.5 1 ppm[a]	pH = 4.5 0 ppm[a]
Sterile	286.20	16.34	620.66	32.03
	840.31	40.22	6.87	143.96
Nonsterile	26.70	7.18	10.69	6.48
	177.70	18.05	511.73	2.90
	156.82	3.24	148.50	4.36
Bacterial	2.00	5.91	5.43	56.72

(a) = Oxygen Concentration of Elution Medium

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groups of bacteria are nutritionally versatile chemoheterotrophs. The acinetobacter group tend to be oxidase negative, whereas the pseudomonads tend to have both oxidase positive and negative members. The acinetobacter group are non-motile and tend to be dominated by the motile pseudomonads under conditions of low oxygen. However, under favourable oxygen conditions the acinetobacter will out-compete the pseudomonads.

The different growth patterns that these two organisms display under decreasing oxygen levels may explain the change in dominance observed during the experiment. The observations suggest that the initial oxygen levels in the tailings were relatively high, supporting the growth of the acinetobacter. As the oxygen concentration was decreased to 1.0 ppm and below, the pseudomonads became the dominant group in the tailings. At this time, it is not possible to quantify this result as no attempt was made to count the various isolates. Future investigations into the relative change in the growth of these bacteria may be warranted.

The number of colony forming units per gram of tailings (CFU/g) was also determined during the experiment (Table 3.29). In general, a greater number of bacteria were found in the samples taken from the top of the bacterial columns than from either the middle or bottom of the columns. However, there were no differences observed that could be attributed to changes in pH or oxygen conditions.

3.4.2 Visual Analysis of the Uranium Mill Tailings

A non-sterile column of uranium mill tailings from each treatment was opened at the conclusion of the experiment. The tailings exposed to the anaerobic medium showed black rings at the bottom of the columns and was possibly evidence that the bottoms of the columns had become sufficiently reduced to produce H_2S (Figure 3.2). However, no smell attributed to H_2S was detected. Furthermore, the zone was located below the bacterial-sampling ports and would not have been detected during the

Table 3.29 Number of Colony Forming Bacteria in Anaerobic and Near-Anaerobic Uranium Mill Tailings

Position in Tailings Column	Week Sampled	CFU/g[a] of Uranium Mill Tailings pH = 3.5 1 ppm[b]	pH = 3.5 0 ppm[b]	pH = 4.5 1 ppm[b]	pH = 4.5 0 ppm[b]
Top	0	7.3×10^5	TNTC[c]	2.55×10^5	7.7×10^5
	2	3.0×10^5	5.9×10^5	1.0×10^7	1.7×10^6
	4	6.4×10^4	2.6×10^6	7.1×10^4	1.1×10^5
	6	5.7×10^6	8.49×10^5	1.8×10^5	9.3×10^2
	8	3.5×10^6	ND[d]	2.0×10^7	9.0×10^4
Middle	0	4.2×10^5	1.2×10^6	2.8×10^3	8.9×10^5
	2	4.2×10^5	1.4×10^4	3.0×10^6	1.6×10^5
	4	2.4×10^5	7.6×10^6	1.2×10^7	7.2×10^3
	6	1.1×10^7	2.97×10^4	4.14×10^4	3.0×10^6
	8	1.4×10^5	1.7×10^5	ND[d]	9.6×10^4
Bottom	0	1.84×10^5	6.9×10^5	ND[d]	ND[d]
	2	3.07×10^3	1.4×10^4	1.6×10^6	2.17×10^5
	4	1.3×10^5	1.4×10^5	ND[d]	2.6×10^4
	6	1.7×10^6	1.5×10^5	2.01×10^4	1.7×10^5
	8	8.1×10^6	7.0×10^4	1.5×10^4	6.8×10^6

(a) CFU/g = Colony forming units per gram of tailings

(b) Oxygen Concentration of Elution Medium

(c) TNTC = The number of colonies on the 10^5 dilution plate were to numerous to be counted

(d) ND = No bacteria detected at 10^2 dilution

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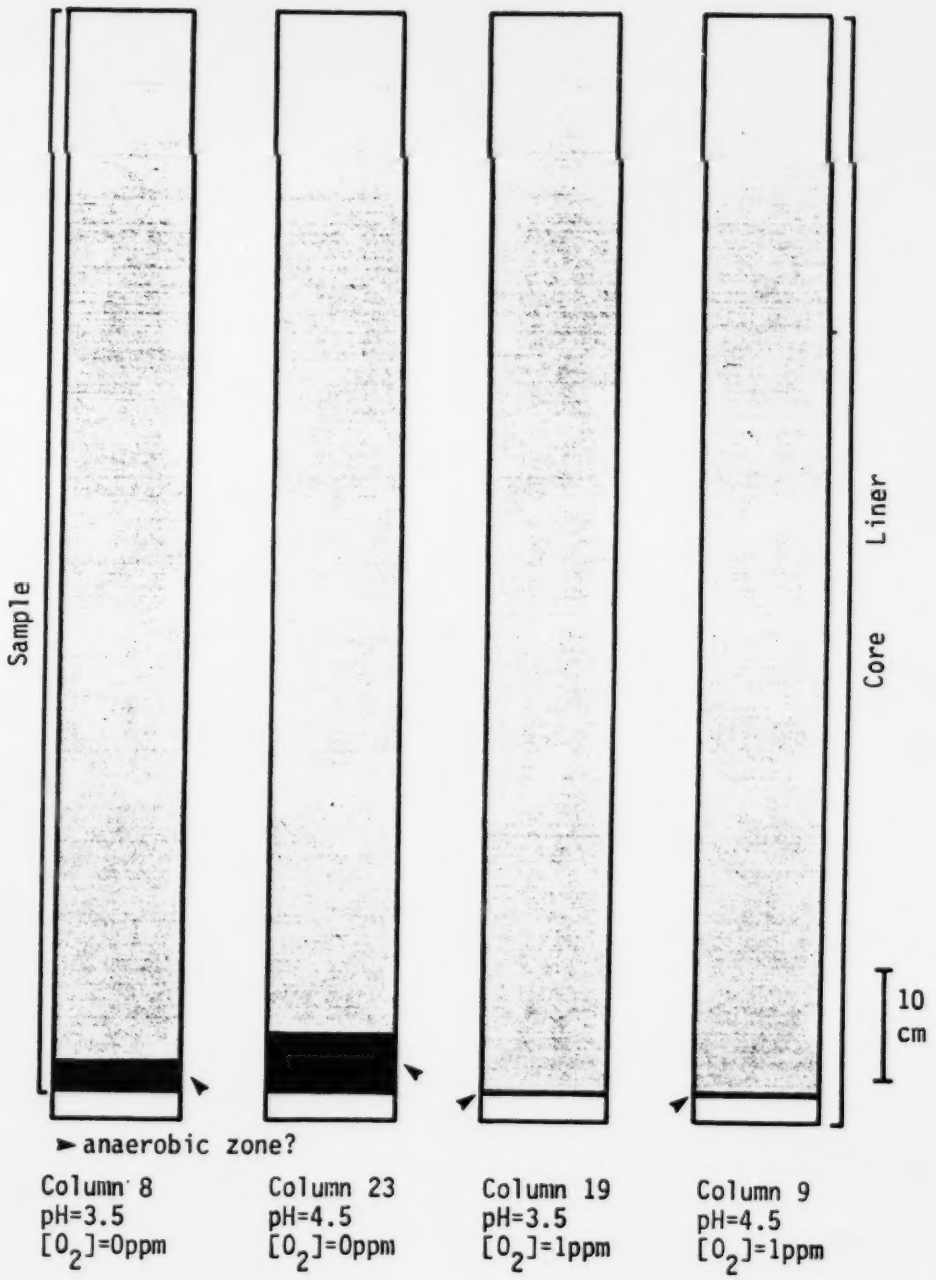

Figure 3.2. A diagram representing photographs of non-sterile columns at the conclusion of the experiment.

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experiment. The observations suggest that the experiment required a significantly longer duration. The results of this experiment represented changes over a relatively short period of time.

3.5 RADIUM-266 AND LEAD-210 ANALYSES

Approximately 1 L of eluate was collected from each of the columns for Pb-210 and Ra-226 analyses. The volumne of the eluates were reduced to aproximately 50 mL, acidified (HCl) and sent to Dr. H. Sharma (University of Waterloo) for analysis.

The results of these analyses have been corrected for the original volume of the eluate and are presented in Tables 3.30 to 3.37. No trends in the concentration of Pb-210 or Ra-226 could be described resulting from changes in either pH or oxygen concentration. There was also no evidence that the bacteria influenced the mobility of radiocations. However, a decrease in the concentrations of these metals over the duration of the experiment was observed, probably resulting from a depletion of the radiocations in the tailings.

The concentration of lead leached from the uranium mill tailings was between two and three orders of magnitude greater than the concentration of radium.

It is known that the distribution and precipitation of radium is often controlled by the chemistry of barium. The precipitation of barium sulphate coprecipitates radium and the subsequent radiocation release is in proportion to the disolution of this compound, while both barium and lead sulphates are only very slightly soluble. The solubility of lead sulphate is significantly higher and may account for the variations observed.

Table 3.30 Lead-210 Eluted from the Uranium Mill Tailings - First Sample

Treatment	Lead-210 Concentration (pCi/L) x 10^3 pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.16	0.63	1.32	0.40
	0.21	0.52	0.19	0.99
Nonsterile	0.22	0.85	0.22	0.05
	0.18	0.67	0.85	0.11
	1.12	1.20	1.22	1.32
Bacterial	0.10	0.47	NC	0.75

NC = No sample collected

Table 3.31 Lead-210 Eluted from the Uranium Mill Tailings - Second Sample

Treatment	Lead-210 Concentration (pCi/L) x 10^3 pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.18	1.50	0.35	NC
	NC	0.39	0.23	NC
Nonsterile	NC	0.62	0.30	1.29
	0.30	0.67	0.28	0.14
	NC	0.60	0.94	NC
Bacterial	0.22	1.18	1.06	0.94

NC = No sample collected

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Table 3.32 Lead-210 Eluted from the Uranium Mill Tailings - Third Sample

Treatment	Lead-210 Concentration (pCi/L) x 10^3 pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	0.62	1.92	1.34
	0.05	0.52	NC	1.33
Nonsterile	0.20	0.94	0.12	0.41
	0.15	0.09	NC	0.12
	0.76	1.30	NC	1.13
Bacterial	NC	NC	NC	1.06

NC = No sample collected

Table 3.33 Lead-210 Eluted from the Uranium Mill Tailings - Fourth Sample

Treatment	Lead-210 Concentration (pCi/L) x 10^3 pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	0.76	0.83
	0.11	0.47	NC	0.25
Nonsterile	0.17	0.90	0.16	NC
	0.23	0.67	0.17	0.12
	0.26	0.87	1.15	0.90
Bacterial	NC	NC	0.60	NC

NC = No sample collected

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Table 3.34 Radium-226 Eluted from the Uranium Mill Tailings - First Sample

Treatment	Radium-226 Concentration (pCi/L) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	4.60	ND	ND	3.63
	18.68	41.52	32.46	11.22
Nonsterile	ND	72.92	3.27	ND
	2.33	ND	29.99	4.62
	ND	3.08	ND	8.04
Bacterial	11.67	31.26	NC	5.02

NC = No sample collected
ND = Below detection limits of procedure

Table 3.35 Radium-226 Eluted from the Uranium Mill Tailings - Second Sample

Treatment	Radium-226 Concentration (pCi/L) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	ND	22.34	2.12	NC
	NC	ND	57.99	NC
Nonsterile	NC	ND	39.67	18.25
	ND	4.74	ND	10.23
	NC	3.77	ND	NC
Bacterial	28.15	110.53	135.71	25.76

NC = No sample collected
ND = Below detection limits of procedure

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Table 3.36 Radium-226 Eluted from the Uranium Mill Tailings - Third Sample

Treatment	Radium-226 Concentration (pCi/L) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	62.90	61.11	ND
	ND	43.15	NC	2.06
Nonsterile	2.44	ND	ND	ND
	ND	ND	NC	24.78
	ND	13.00	NC	10.12
Bacterial	NC	NC	NC	12.80

NC = No sample collected
ND = Below detection limits of procedure

Table 3.37 Radium-226 Eluted from the Uranium Mill Tailings - Fourth Sample

Treatment	Radium-226 Concentration (pCi/L) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	3.64	4.36
	7.73	78.60	NC	13.44
Nonsterile	ND	ND	4.18	NC
	19.96	ND	ND	20.80
	ND	ND	ND	ND
Bacterial	NC	NC	5.84	NC

NC = No sample collected
ND = Below detection limits of procedure

4.0 CONCLUSIONS

(1) The bacteria play a role in the chemistry of the mill tailings as supported by the results presented in the above sections. However, the magnitude of the impact of the bacteria on the uranium mill tailings was trivial (approximately 1%) when compared to chemical reactions.

(2) Visual examination of the uranium mill tailings suggested that the experiment should have been run over a considerably longer period of time (approx. 1 y) to obtain more meaningful results. It is possible that with time the importance of bacterial impact increases.

5.0 REFERENCES

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Raw Data Nutrient Analyses

Table A1 pH of First Sample Elutions

Treatment	pH of Samples pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	4.08	2.14	4.42	3.39
	5.85	3.98	4.31	3.82
Nonsterile	3.20	3.24	4.13	3.86
	3.08	3.56	4.26	3.70
	3.27	3.65	2.16	3.38
Bacterial	3.49	4.61	2.63	3.54

Table A2 pH of Second Set of Elutions

Treatment	pH of Samples pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	4.52	2.72	5.13	NC
	NC	4.15	4.05	NC
Nonsterile	NC	3.54	4.83	3.98
	3.53	3.75	4.95	3.89
	NC	3.69	2.86	NC
Bacterial	3.83	4.41	3.00	3.88

NC = No Sample Collected

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Table A3 pH of Third Set of Elutions

Treatment	pH of Samples			
	pH = 3.5		pH = 4.5	
	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	3.06	7.62	3.31
	5.72	4.21	4.05	3.70
Nonsterile	3.84	3.69	6.57	4.80
	4.07	4.10	7.37	3.62
	3.87	4.90	NC	5.79
Bacterial	NC	NC	NC	4.06

NC = No Sample Collected

Table A4 pH of Fourth Set of Elutions

Treatment	pH of Samples			
	pH = 3.5		pH = 4.5	
	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	NC	6.44	3.35
	6.25	3.52	NC	5.49
Nonsterile	4.71	3.71	6.37	NC
	3.96	3.72	5.92	3.55
	5.09	3.54	3.16	3.73
Bacterial	NC	NC	3.73	NC

NC = No Sample Collected

Table A5 Volume of First Sample

Treatment	Volume of Samples (mL) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	1590.00	383.00	1616.00	1686.00
	1535.00	1171.00	521.00	1482.00
Nonsterile	1139.00	826.00	1580.00	846.00
	690.00	1379.00	1055.00	389.00
	1430.00	1296.00	397.00	1029.00
Bacterial	878.00	737.00	153.00	1058.00

Table A6 Volume of Second Set of Samples

Treatment	Volume of Samples (mL) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	359	330	1037	NC
	NC	359	1037	NC
Nonsterile	NC	824	1037	273
	1062	1037	688	1037
	NC	1006	472	NC
Bacterial	1050	216	197	286

NC = No Sample Collected

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Table A7 Volume of Third Set of Samples

Treatment	Volume of Samples (mL)			
	pH = 3.5		pH = 4.5	
	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	446	228	799
	1050	755	NC	1050
Nonsterile	673	343	720	582
	609	825	76	666
	1050	711	62	926
Bacterial	NC	89	19	540

NC = No Sample Collected

Table A8 Volume of Fourth Set of Samples

Treatment	Volume of Samples (mL)			
	pH = 3.5		pH = 4.5	
	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	NC	NC	1100	1100
	1100	446	NC	1064
Nonsterile	1100	310	1050	NC
	1100	530	1100	1058
	1100	458	1050	363
Bacterial	NC	NC	1050	NC

NC = No Sample Collected

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Table A9 Total Iron, Analyses from First Sample

Treatment	Total Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	1.3	136	0.89	31
	0.07	3.3	2.7	2.4
Nonsterile	0.79	159	4.5	0.38
	2.4	85	2.5	1.7
	1	4.3	685	244
Bacterial	2.6	0.1	80	150

Table A10 Total Iron, Analyses from Second Set of Samples

Treatment	Total Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.07	88	ND	NC
	NC	2.06	0.26	NC
Nonsterile	NC	4.34	ND	0.85
	1.61	3.97	0.25	0.34
	NC	4.75	27	NC
Bacterial	0.84	2.79	28	0.32

NC = No Sample Collected
ND = Below Detection Limit of Method

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Table A11 Total Iron, Analyses from Third Set of Samples

Treatment	Total Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	4.2	ND	1.43
	ND	0.15	NC	0.04
Nonsterile	4.7	1.05	0.68	0.57
	0.15	0.13	ND	0.81
	0.04	0.56	55	3.3
Bacterial	NC	NC	NC	0.24

NC = No Sample Collected
ND = Below Detection Limit of Method

Table A12 Total Iron, Analyses from Fourth Set of Samples

Treatment	Total Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	1.8
	ND	ND	NC	0.16
Nonsterile	3.5	1.5	ND	NC
	1.5	ND	ND	0.82
	0.46	0.78	3.6	3.6
Bacterial	NC	NC	7.3	NC

NC = No Sample Collected
ND = No Iron Detected

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Table A13 Ferrous Iron, Analyses from First Sample

Treatment	Ferrous Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.6	5.9	0.55	5.8
	0.012	3.9	3.1	1.9
Nonsterile	0.12	0.026	0.31	0.3
	0.45	0.73	0.15	0.2
	0.25	1.4	4.7	1.4
Bacterial	0.17	0.39	0.14	3.4

Table A14 Ferrous Iron, Analyses from Second Set of Samples

Treatment	Ferrous Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.029	>10	0.048	NC
	NC	0.19	0.14	NC
Nonsterile	NC	0.25	0.016	0.25
	0.13	0.34	0.15	0.051
	NC	0.39	3.7	NC
Bacterial	0.08	0.077	0.82	0.054

NC = No Sample Collected
>10 = Greater than 10 ppm

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Table A15 Ferrous Iron, Analyses from Third Set of Samples

Treatment	Ferrous Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.069	0.112	ND	0.034
	ND	0.109	0.018	NC
Nonsterile	0.137	0.014	ND	0.021
	ND	ND	0.050	0.004
	0.049	0.100	NC	0.095
Bacterial	NC	NC	NC	0.017

NC = No Sample Collected
ND = Below Detection Limit of Method

Table A16 Ferrous Iron, Analyses from Fourth Set of Samples

Treatment	Ferrous Iron Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	0.175
	ND	ND	NC	0.108
Nonsterile	0.037	0.004	ND	NC
	0.053	ND	ND	0.035
	ND	0.049	0.079	0.047
Bacterial	NC	NC	0.183	NC

NC = No Sample Collected
ND = Below Detection Limit of Method

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Table A17 Phosphate Analyses from First Sample

Treatment	Phosphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	0.18	0.03	0.032	0.019
	ND	0.031	0.0094	ND
Nonsterile	0.0069	0.0025	ND	0.0062
	ND	0.0087	0.0062	ND
	0.0062	0.0025	0.00062	ND
Bacterial	ND	0.0062	0.021	0.048

ND = Below Detection Limit of Method

Table A18 Phosphate Analyses from Second Set of Samples

Treatment	Phosphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	ND	0.0147	0.1828	NC
	NC	0.0036	0.0019	NC
Nonsterile	NC	0.0062	0.0019	0.0045
	0.0045	0.0019	0.0028	0.0028
	NC	ND	0.228	NC
Bacterial	0.0019	0.0062	0.0054	ND

ND = Below Detection Limit of Method
NC = No Sample Collected

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Table A19 Phosphate Analyses from Third Set of Samples

Treatment	Phosphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	ND
	0.3583	ND	NC	0.1371
Nonsterile	0.028	ND	NC	ND
	ND	ND	0.006	ND
	ND	ND	NC	ND
Bacterial	NC	NC	NC	0.011

NC = No Sample Collected
ND = Below Detection Limit of Method

Table A20 Phosphate Analyses from Fourth Set of Samples

Treatment	Phosphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	0.3449	ND
	0.4219	0.0059	NC	NC
Nonsterile	ND	ND	0.0083	NC
	0.1572	0.0077	0.4571	ND
	0.1345	ND	0.0387	0.012
Bacterial	NC	NC	0.0011	NC

ND = Below Detection Limit of Method
NC = No Sample Collected

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Table A21 Nitrate Analyses from First Sample

Treatment	Nitrate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	29	ND	22	22
	24	15	10	27
Nonsterile	15	2.3	20	13
	23	12	16	20
	24	21	1.5	9.5
Bacterial	23	16	ND	12

ND = Below Detection Limit of Method

Table A22 Nitrate Analyses from Second Set of Samples

Treatment	Nitrate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	66.7	ND	52.3	NC
	NC	56.9	51.4	NC
Nonsterile	NC	60.8	35.1	60
	65.7	52.3	42.6	55.5
	NC	NR	28.5	NC
Bacterial	67.2	55.2	11	48.5

NC = No Samples Collected
NR = No Value Reported
ND = Below Detection Limit of Method

Table A23 Nitrate Analyses from Third Set of Samples

Treatment	Nitrate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	ND	ND	25.8
	12.5	22.8	NC	15.9
Nonsterile	26.08	21.4	NC	31.7
	25	14.8	3.33	32.4
	19.4	14.9	NC	13.1
Bacterial	NC	NC	NC	28.1

NC = No Sample Collected
NR = No Value Reported
ND = Below Detection Limit of Method

Table A24 Nitrate Analyses from Fourth Set of Samples

Treatment	Nitrate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	ND	11.16
	30.23	26.24	NC	NR
Nonsterile	28.33	17.43	3.35	NC
	26.33	8.07	24.6	32.69
	22.79	15.07	19.06	16.61
Bacterial	NC	NC	15.25	NC

NC = No Sample Collected
NR = No Value Reported
ND = Below Detection Limit of Method

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Table A25 Sulphate Analyses from First Sample

Treatment	Sulphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	430	1900	560	870
	670	870	870	720
Nonsterile	870	820	560	670
	970	970	720	560
	800	560	2300	1100
Bacterial	820	410	1400	1400

Table A26 Sulphate Analyses from Second Set of Samples

Treatment	Sulphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	870	900	780	NC
	NC	960	420	NC
Nonsterile	NC	870	650	1600
	3800	1100	580	800
	NC	840	870	NC
Bacterial	1500	920	580	540

NC = No Sample Collected

Table A27 Sulphate Analyses from Third Set of Samples

Treatment	Sulphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	1390	1110
	517	995	NC	397
Nonsterile	585	750	NC	925
	745	227	1170	652
	517	727	NC	682
Bacterial	NC	NC	NC	1280

NC = No Sample Collected

Table A28 Sulphate Analyses from Fourth Set of Samples

Treatment	Sulphate Concentration (ppm) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	1110	1810
	601	590	NC	258
Nonsterile	923	1120	429	NC
	490	1090	441	1070
	1090	1020	1020	585
Bacterial	NC	NC	1750	NC

NC = No Sample Collected

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Bacterial Characterization Results
from Enterotube/Oxiferm
Rapid Identification System

Appendix 2

Pre-Experiment Collection

PLATE IDENTITY	C18,T14b	C18,T14b	C18,T14b	C18,T8a	C4,T4	C4,T10	C4,T13	C24,T5	C24,T3	C12,T6	C12,T1	C12,T2	C12,T2
Colony Morphology	Small White	Medium White	Large White	Medium White Mucoid	Medium White Mucoid	Large White Mucoid	Medium White Mucoid	Medium White	Small White	Medium White	Small White Mucoid	Medium White	Large White
Cell Shape	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram RX	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidaze RX	-	-	+	-	-	-	-	-	-	-	-	-	$^{-}/_{+}$
Glucose/Gas	$^{-}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$	$^{o}/_{-}$
LYS	-	-	-	-	-	-	-	-	-	-	-	-	-
ORN	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S/IND	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$	$^{-}/_{-}$
ADON	-	-	-	-	0	-	-	-	-	-	-	-	-
LAC	-	-	-	-	0	-	-	-	-	-	-	-	-
ARAB	-	-	-	-	-	-	-	-	-	-	-	-	-
SORB	-	-	-	-	0	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	$^{-}/_{-}$
DUL/Pa	$^{-}/_{+}$*	$/_{+}$*	$^{-}/_{+}$*	$^{-}/_{-}$	$^{-}/_{-}$	-	$^{-}/_{+}$*	$^{-}/_{-}$	$^{-}/_{+}$*	$^{-}/_{+}$*	-	$^{-}/_{+}$*	$^{-}/_{+}$*
UREA	-	+	+*	+	+	-	+	+	+	+	-	+*	-
CIT	-	G^{+}	+	G^{+}	G^{+}	-	-	G^{+}	G^{+}	G^{+}	-	+	+
Identity Letter	A	B	C	D	E	F	G	H	I	J	A[1]	B	C[1]
Anaerobic Dextrose			$^{+}/_{-}$										+
ARG			-										-
N_2			-										-
H_2S/IND			-										-
XYL			-										-
Aerobic Dextrose			+										-
UREA			+										-
CIT			+										+

* Very Weak
rod
range
reen

DOBROCKY SEATECH

1st Collection

PLATE IDENTITY	C4,T24	C4,T24	C4,T22	C4,T18	C12,T25	C12,T25	C12,T27	C12,T23	C18,T28	C18,T21	C18,T21	C18,T21	C18,T29	C24,T26	C24,T26	C24,T26	C24,T19	C24,T17	C24,T17
Colony Morphology	Yellow Mucoid	White Mucoid	Yellow Mucoid	Large White Mucoid	Large White Mucoid	Small White Mucoid	Yellow Mucoid	Small White	Yellow Mucoid	White Mucoid	Large White Mucoid	Small Yellow	White Mucoid	White Mucoid	Small Yellow	Large Yellow	Yellow Mucloid	Large Yellow	Small White
Cell Shape	R	R	R	R	R	R	R	R	R	C	R	C	C	R	C	C	R	C	R
Gram RX	-	-	-/+	-	+/-	-	-	-	-	+	-	+	+	-	+	+	-	+	-
Oxidase RX	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Glucose/Gas	+	-	-	+	-	+	+	-	-	+	+	+	+	+	-	+	+	-	+
LYS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ORN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S/IND	-/-	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
ADON	-	-	-	-	-	-	-	-	-	-	B	-	-	-	-	P	+	-	-
LAC	+	+/-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-
ARAB	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-
SORB	-	-	-	-	-	-	-	-	-	+	B	-	-	-	-	-	+	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DUL/Pa	-/-	-/+	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
UREA	+	-	+	+	-	+	+	-	-	+	+	-	+	+	-	-	+	-	+
CIT	-	+	-	+	+	+	+	-	+	-	+	-	-	+	-	-	+/-	-	+
Identity Letter	A	B	C	D	E	F	G	H	I	J	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1	J^1
Anaerobic Dextrose					-				-										
ARG					-				-										
N_2					-				-										
H_2S/IND					-/-				-/-										
XYL					+				+										
Aerobic Dextrose					+				+										
UREA					-				-										
CIT					+				+										

R = rod
C = cocc
B = Blue

DOBROCKY SEATECH

2nd Collection

PLATE IDENTITY	C24,T2	C24,T6	C24,T6	C4,T3	C18,T13	C18,T13	C18,T13	C24,T5	C4,T18	C4,T18	C4,T18	C12,T15	C24,T4	C4,T11	C12,T2
Colony Morphology	Small White	Large Mucoid	Small White	Large Mucoid	Medium Yellow	Small White	Large Mucoid	Large Mucoid	Large Mucoid	Small Mucoid	Small White	Small White	Small White	Small White	Small White
Cell Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram RX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidaze RX	-	+	-	+	+	+	+	-	+	+	-	-	-	-	-
Glucose/Gas	-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-	+/-	+/-	+/-	+/-	+/-
LYS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ORN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S/IND	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
ADON	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ARAB	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
SORB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DUL/Pa	-	-/-	-/-	-/-	-/-	-/-	-/-	-	-	-	-/-	-/-	-/-	-/-	-/-
UREA	-	-	+	-	-	-	-	+	-	-	+	-	+	+	+
CIT	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Identity Letter	A	B	C	D	E	F	G	H	I	J	A^1	B^1	C^1	D^1	E^1
Anaerobic Dextrose		-		-	-	-	-		-	-					
ARG		-		-	-	-	-		+	-					
H_2		-		-	-	-	-		-	-					
H_2S/IND		-/-		-/-	-/-	-/-	-/-		-/-	-/-					
XYL		+		+	+	-	-		+	+					
Aerobic Dextrose		+		+	+	+	+		+	+					
UREA		+		-	+	-	-		-	-					
CIT		+		+	+	+	+		+	+					

3rd Collection

PLATE IDENTITY	C18T4-1	C24T15-1	C24T15-2	C24T15-3	C24T12-1	C24T12-2	C4T9-1	C4T9-2	C4T9-3	C4T7-1	C4T7-2	C4T7-3	C4T1-1	C4T1-2	C4T1-3	C4T1-4	C18T5-1	C18T5-2	C18T5-3
Colony Morphology	Medium White	Medium White	Hard White	White Mucoid	White Mucoid	Small White	Large White Mucoid	Small Yellow	Yellow Mucoid	Large Mucoid	Medium Yellow	Small White	Yellow	Small White	Large White Mucoid	Medium White Mucoid	Large Yellow	Medium White Mucoid	Large White Mucoid
Cell Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram RX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase RX	-	+	-	-	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+
Glucose/Gas	+/-	+/-	+/-	+/-	-	-	-	-	-	-	-	-	-	+	-	+	+/-	-	-
LYS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ORN	-	-	-	-	-	-/	-/	-	-/	-	-	-	-	-	-	-	-	-	-
H_2S/IND	-	-	-	-	-	-	-	-/	-	-	-	-	-	-	-	-	-	-	-
ADON	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ARAB	-	+	+	+	+	-	-	+	+	-	+	-	+	-	+	+	+	+	-
SORB	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-		0	-	-	-	-	-	-	-	-	-	-	-
DUL/Pa	-	-/-	-/-	-	-/+	-/-	-/-	-/-	-/-	-	-	-	-	-	-	-	-	-	-
UREA	+	-	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	+	
CIT	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+
Identity Letter	A	B	C	D	E	F	G	H	I	J	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
Anaerobic Dextrose		-			-		-	-	-	-	-		-	-	-	-	-	-	-
ARG		+			-		+	+	-	-	-		+	-	+	+	-	-	-
N_2		-			+		-	-	+	-	-		-	-	-	-	-	+	-
H_2S/IND		-			-		-	-	-	-	-		-	-	-	-	-	-	-
XYL		+			+		+	+	+	+	+		+	-	+	+	-	+	+
Aerobic Dextrose		+			+		+	+	+	+	+		+	-	+	+	+	+	+
UREA		-			-		-	-	-	-	-		+	+	+	+	-		-
CIT		+			-		+	+	+	+	+		+	-	+	+	-	+	+

R = rod

O = orange

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4th Collection

PLATE IDENTITY	C4T10-1	C4T10-2	C4T10-3	C4T3-1	C4T3-2	C4T3-3	C4T11-1	C12T1-1	C12T1-2	C12T1-3	C12T15-1	C18T2-1	C18T2-2	C24T12-1
Colony Morphology	Small White	Large White	Small Yellow	Large Yellow	Large White	Small White	Medium Yellow	Hard White	Large White Mucoid	Small White	Medium Yellow	Large White Mucoid	Medium Yellow	Large Yellow Mucoid
Cell Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram RX	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Oxidaze RX	-	-	+	+	+	-	-	+	+	+	-	+	-	+
Glucose/Gas	+/-	-/-	-/-	-/-	-/-	+/-	+/+	-/-	-/-	+/-	-/-	+/-	-/-	-/-
LYS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ORN	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S/IND	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
ADON	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ARAB	-	-	+	+	-	-	-	-	-	+	-	-	-	+
SORB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DUL/Pa	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/+	-/+	-/+	-/+	/+	/+	/+
UREA	+	+	-	-	-	+	-	+	+	-	-	-	-	-
CIT	-	-	+	+	+	-	+	+	+	+	+	+	+	+
Identity Letter	A	B	C	D	E	F	G	H	I	J	A[1]	B[1]	C[1]	D[1]
Anaerobic Dextrose			-	-	-	-		-	-	-		-		
ARG			-	-	-			-	-	-		-		
N_2			+	-	-			+	+	-				
H_2S/IND			-/-	-/-	-/-			-/-	-/-	-/-		-/-		
XYL			+/-	+	+			-	-	+		+		
Aerobic Dextrose			+	+	+			+	+	+		+		
UREA			-	-	+			+	-	-		-		
CIT			+	+	+			+	+	+		+		

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Lead-210 and Radium-226 Data as Reported by

Dr. H. Sharma, University of Waterloo

Appendix 3

Table A3.1 Lead-210 Eluted from the Uranium Mill Tailings - First Sample

Treatment	Lead-210 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	213[a]	101	1783	565
	254	447	44	1240
Nonsterile	189	244	285	73
	166	750	643	84
	1255	1212	126	987
Bacterial	58	218	NC	567

(a) Detection limit is 2 pCi
NC = No sample collected

Table A3.2 Lead-210 Eluted from the Uranium Mill Tailings - Second Sample

Treatment	Lead-210 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	178[a]	281	344	NC
	NC	87	227	NC
Nonsterile	NC	416	293	162
	277	648	153	134
	NC	526	310	NC
Bacterial	213	90	74	143

(a) Detection limit is 2 pCi
NC = No sample collected

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Table A3.3 Lead-210 Eluted from the Uranium Mill Tailings - Third Sample

Treatment	Lead-210 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC[a]	192	207	909
	46	326	NC	1289
Nonsterile	108	197	69	112
	73	64	NC	72
	661	753	NC	884
Bacterial	NC	NC	NC	355

(a) Detection limit is 2 pCi
NC = No sample collected

Table A3.4 Lead-210 Eluted from the Uranium Mill Tailings - Fourth Sample

Treatment	Lead-210 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	773	845
	110	143	NC	230
Nonsterile	121	150	146	NC
	230	259	156	111
	267	269	1097	198
Bacterial	NC	NC	571	NC

(a) Detection limit is 2 pCi
NC = No sample collected

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Table A3.5 Radium-226 Eluted from the Uranium Mill Tailings - First Sample

	Radium-226 (pCi)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	6.0	ND	ND	5.1
	23.2	36.0	7.4	14.0
Nonsterile	ND	21.0	4.2	ND
	2.1	ND	33.5	3.5
	ND	3.1	ND	6.0
Bacterial	7.0	14.6	NC	3.8

NC = No sample collected
ND = Below detection limit of 2 pCi

Table A3.6 Radium-226 Eluted from the Uranium Mill Tailings - Second Sample

	Radium-226 (pCi)			
	pH = 3.5		pH = 4.5	
Treatment	1 ppm	0 ppm	1 ppm	0 ppm
Sterile	ND	4.2	2.1	NC
	ND	ND	57.0	NC
Nonsterile	NC	ND	39.0	9.5
	ND	4.6	ND	10.0
	NC	3.3	ND	NC
Bacterial	27.0	8.4	9.5	8.5

NC = No sample collected
ND = Below detection limit of 2 pCi

Table A3.7 Radium-226 Eluted from the Uranium Mill Tailings - Third Sample

Treatment	Radium-226 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	19.5	6.6	ND
	ND	27.1	NC	2.0
Nonsterile	1.3	ND	ND	ND
	ND	ND	NC	14.3
	ND	ND	NC	7.9
Bacterial	NC	NC	NC	4.3

NC = No sample collected
ND = Below detection limit of 2 pCi

Table A3.8 Radium-226 Eluted from the Uranium Mill Tailings - Fourth Sample

Treatment	Radium-226 (pCi) pH = 3.5 1 ppm	pH = 3.5 0 ppm	pH = 4.5 1 ppm	pH = 4.5 0 ppm
Sterile	NC	NC	4.0	4.8
	8.5	23.9	ND	14.3
Nonsterile	ND	ND	3.9	NC
	20.4	ND	ND	18.8
	ND	ND	ND	ND
Bacterial	NC	NC	6.0	NC

NC = No sample collected
ND = Below detection limit of 2 pCi

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Thorium Analyses

Appendix 4

Leachates collected from uranium mill tailings, eluted with solutions of differing pH and oxygen concentration, were analyzed for their Thorium-230 concentrations (Table 4.1). The leachates were collected after the columns had been eluted for eight weeks. The Th-230 was determined according to the following method reported by Chemex Ltd.

All the samples were filtered through a 0.45 μm cellulose acetate filter prior to analysis. Lanthanum chloride was added as a carrier for the Thorium and Barium Nitrate was added as a holdback carrier for radium isotopes. Thorium was coprecipitated with Lanthanum Hydroxide, purified and coprecipitated with Lanthanum Fluoride. The final precipitate was collected on a Tuffryn filter (0.2 μm) for alpha counting. The alpha spectroscopy measurement was performed using an Ortec Model 496 alpha spectrometer. The activity of the samples was compared with activities of the standard Th-230 solution obtained from Isotope Products Ltd. of Burbank, California. This Th-230 stock solution is traceable to the U.S. National Bureau of Standards and is certified to have an uncertainty not exceeding ± 5.0% at the 99% confidence interval level.

The concentration of Th-230 in the leachates was quite low and in many cases not detectable (Table 4.1). There tended to be more Th-230 found in the leachates from the nonsterile and anaerobic tailings. These results suggest that the presence of bacteria and anaerobic conditions may have increased the mobility of the Th-230 in the uranium mill tailings. The effect of the bacteria on the mobility of the Th-230 was probably not direct but due to more anaerobic conditions created as a function of the bacteria activity. The increase in the mobility of the Th-230 is consisent with previously stated hypothesis that anaerobic conditions increase the mobility of Th-230 in the tailings.

The samples for Th-230 were not taken until after the tailings had undergone extensive leaching. In future studies it may be adviseable to allow the anaerobic conditions to develop in some of the tailings columns prior to beginning the leaching process. This would provide a better comparison of the effects of anaerobic conditions on the mobility of Th-230.

In summary, the data collected during this experiment indicate that anaerobic conditions in the uranium mill tailings increase the concentration of Th-230 in the eluate and that the bacteria present in the uranium mill tailings through normal metabolic activity can promote the conditions which will lead to increased Th-230 mobility.

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Table 4.1 Thorium-230 Analysis from Uranium Mill Tailings

	Th-230 Concentration Bq/L			
	pH 3.5		pH 4.5	
	1.0 ppm	0 ppm	1.0 ppm	0 ppm
Sterile		0.05 ± 0.05[ab]	<0.05[c]	0.05 ± 0.05
		<0.05		
Nonsterile	<0.05		0.10 ± 0.05	0.10 ± 0.05
	<0.05[d]			
	0.10 ± 0.05	0.12 ± 0.07	1.01 ± 0.15	<0.05
	<0.05[d]		<0.05[d]	
	0.05 ± 0.05	0.05 ± 0.05		
	0.05 ± 0.05[d]	0.45 ± 0.10[d]		
Bacterial				0.15 ± 0.05

(a) Samples at detection limit.

(b) Thorium concentration ± 95% confidence limit.

(c) Below detection limit.

(d) Replicate samples from the columns were submitted for analysis as completely separate samples.